

WORKSHOP ON RIBOSOMES AND THEIR ROLE IN DISEASE

National Institute of Diabetes and Digestive and Kidney Diseases

Hilton Washington D.C./Rockville Hotel and Executive Conference Center, Rockville, MD

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With the recent discovery of a ribosomal protein defect in some cases of Diamond-Blackfan Anemia, exploration into how the ribosomes could cause this disease, as well as others, needs to take place. To meet this need, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) at the National Institutes of Health (NIH), in collaboration with the National Heart, Lung, and Blood Institute (NHLBI) and the Centers for Disease Control and Prevention (CDC), sponsored a public workshop on the role of ribosomal biogenesis in disease. Experts in the field presented their findings during this 2-day workshop, reviewed what's known about ribosomal biogenesis, and then embarked on recent clinical connections of ribosomal malfunctioning and disease. The Organizing Committee consisted of Drs. Monica Bessler (Washington University), Terry Rogers Bishop (NIDDK), Steven Ellis (University of Louisville), W. Craig Hopper (CDC), Harvey Lodish (The Whitehead Institute) & Pankaj Qasba (NHLBI). The workshop began with the *overview*, followed by presentations on *the structure & function of the ribosome, the nucleolus & rRNA transcription, ribosomal biogenesis, ribosomes and diseases*, and concluded with the most significant discussion during the meeting, *areas of future research*. This document serves to provide a summary of presentations discussed at the workshop. We begin with "Future Directions" to recapitulate the research study findings.

I. FUTURE DIRECTIONS

Basic Research

- Genotype-Phenotype correlations. Are there correlations of disease severity with deletions or point mutations? What about protein levels?
- Genetics of Diamond-Blackfan Anemia (DBA). Which genes are modifying disease severity? Which genes are involved in predisposition to remission or cancer?
- Ribosomal biology. How are ribosomal protein (RP) genes coordinately regulated? How is subunit stoichiometry regulated? How are biogenesis and signaling affected by a defect in one RP, especially in erythroid cells? Are there tissue-specific characteristics of RP genes? Are RPs functioning in other roles besides being components of the ribosome?
- Model organism development for proteomics and expression studies.

Translational Research

- Assess whether anticipation is occurring in DBA or not. Is the severity of the disease increasing each generation?
- Find the mechanism of spontaneous remission
- Study the roles of ribosomes in hematologic diseases other than DBA.

- Develop human genetic studies, *e.g.*, GWAS studies or development of functional assays for clinical use
- Discover the mechanism of corticosteroid treatment
- Create gene therapy or other novel strategies for treatment of DBA

Train New Research Investigators

II. OVERVIEW OF RESEARCH STUDY FINDINGS

Why Do Mutations in Ribosomal Proteins Mainly Affect Erythropoiesis?

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Why does a defect in ribosome biogenesis lead mainly to a defect in red cell production, macrocytic anemia, and a predisposition to leukemia? Biogenesis of erythrocytes is regulated by many cytokines, extracellular matrix proteins, and other molecules. Formation of erythrocytes from a CFU-E progenitor requires four to five terminal cell divisions over 2 to 3 days, and cells are mainly in the S phase of the cell cycle. Terminal differentiation involves repression of gene transcription, chromatin condensation (possibly similar to that occurring in metaphase chromosomes), nuclear condensation, and enucleation. One possibility for the anemia caused by absence of specific ribosomal proteins is that erythropoiesis, at least in its terminal stages, critically depends on large numbers of ribosomes for translation, and anemia would be caused mainly by insufficient mRNA translation. The resultant reduction in translation of new proteins would slow the terminal cell cycles. Alternatively, or in addition, defects in rRNA/ribosome biogenesis could lead to “nucleolar stress” and directly to defects in cell cycle regulatory proteins within the nucleus. Or increased levels of blocked rRNA processing intermediates and/or ribosome assembly intermediates could lead to defects in chromatin condensation and/or mitosis—possibly due to effects on nucleolar condensation. These effects, which have been demonstrated in other cell lineages, would be exacerbated in erythroid cells because of their amazingly quick cell cycle.

Ribosomal Mutations Cause p53-mediated Dark Skin and Pleiotropic Effects

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Mutations in ribosomal proteins cause the dominantly inherited Minute phenotype in *Drosophila* and mice, and Diamond Blackfan syndrome in humans. During the course of a genetic screen in mice for dark skin (*Dsk*), missense alterations of Ribosomal protein S19 (*Rps19*), and Ribosomal protein S20 (*Rps20*) were identified in the chemically induced mutants *Dsk3* and *Dsk4*, respectively. These mutations act through a common pathophysiologic program in which p53 stabilization causes increased expression of Kit ligand, and consequently, proliferation of epidermal melanocytes. Accumulation of p53 is both necessary and sufficient for the dark skin phenotype but occurs through a paracrine mechanism that depends on keratinocyte-melanocyte interactions. Accumulation of p53 also causes a reduction in body size and erythrocyte count. These results provide a mechanistic

explanation for the diverse collection of phenotypes that accompany reduced dosage of genes encoding ribosomal proteins, and have implications for understanding normal human variation and human disease.

Identification of RPS14 as a 5q⁻ Syndrome Gene by RNA Interference Screen

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Systematic RNA interference (RNAi) to interrogate the function of the genes in the common deleted region was utilized to explore the possible haploinsufficiency mechanism in the 5q⁻ syndrome. Short hairpin RNAs (shRNAs) targeting each of the 41 genes in the region into normal CD34⁺ human bone marrow hematopoietic progenitor cells were introduced to study the effects of each shRNA on hematopoietic differentiation. Knockdown of a single gene RPS14 recapitulated the phenotype of the 5q⁻ syndrome: a block in erythroid differentiation (leading to erythroid cell apoptosis) with relative preservation of megakaryocyte differentiation as measured by FACS analysis. Importantly, forced expression of an RPS14 cDNA in primary bone marrow cells from patients with the 5q⁻ syndrome rescued the phenotype, yet had no effect on cells from myelodysplastic syndrome (MDS) patients without 5q deletions. In addition, RPS14 haploinsufficiency was found to cause a block in the processing of pre-ribosomal RNA and in the formation of the 40S ribosomal subunit.

This ribosomal processing defect is highly analogous to the functional defect seen in Diamond Blackfan Anemia (also characterized by an erythroid differentiation defect and predisposition to acute myelogenous leukemia [AML]), thereby establishing an unexpected link between the molecular pathophysiology of acquired 5q⁻ syndrome and congenital bone marrow failure syndromes. These results indicate that the 5q⁻ syndrome is caused by a defect in ribosomal protein function, highlighting the importance of translational control in hematologic malignancy. The results further suggest that RNAi screening is an effective strategy for identifying causal haploinsufficiency disease genes. Haploinsufficiency of the ribosomal protein encoding the RPS14 gene causes the characteristic hematologic phenotype that defines the 5q⁻ syndrome.

III. STRUCTURE AND FUNCTION OF THE RIBOSOME

Ribosome Biogenesis and the p53 Tumor Suppression Pathway

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Cellular growth and division are inherently disparate processes that are intimately coupled through a delicate network of intracellular signaling programs and extracellular stimulating events. A number of events imparting instability to ribosomal biogenesis can cause nucleolar stress and trigger activation of a p53-dependent checkpoint. A model for a ribosomal biogenesis-p53 checkpoint has been proposed based on the following circumstances: nucleolar stress occurs following inhibition of ribosome

biogenesis either through reduction of rRNA or ribosomal protein; and following nucleolar stress, ribosomal proteins L5, L11, and L23 bind to MDM2 and block MDM2-mediated p53 ubiquitination and degradation, ultimately resulting in activation of a p53-directed cell cycle arrest. According to this model, ribosomal proteins L5, L11, and L23 make up the core response regulators tying together deregulated cellular growth to cell division control. The ribosomal protein-MDM2-p53 pathway provides a molecular switch that may, in fact, constitute a surveillance network monitoring the integrity of ribosomal biogenesis. The MDM2 C4 zinc finger domain has been recently shown to play an important role in this process. Mutations targeting the C4 zinc finger of MDM2 have been reported in human cancers, and now a potential rationale for the occurrence of these mutations in cancer has emerged.

Sdo1p Regulation of Histone Deacetylase Activity in Yeast

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The human disease gene *SBDS* (Shwachman Bodian Diamond syndrome) and its homologs have been implicated in ribosomal assembly and function. To gain insight into their precise molecular functions, a *S. cerevisiae* model of SDS using *SDO1*, the highly conserved yeast ortholog of *SBDS*, was developed to identify small molecules that rescue the slow growth “disease” phenotype of *sdo1* cells, and an unexpected interaction between *SDO1* and histone deacetylases (HDACs) was uncovered: (1) Structurally distinct HDAC inhibitors improved the growth of cells lacking Sdo1p; (2) Overexpression of Sdo1p increased global histone acetylation and ameliorated the toxicity arising from overexpression of the major Class I or II yeast HDACs (*RPD3* or *HDA1*, respectively), but not *SIR2*, the NAD^+ dependent histone deacetylase; (3) Sdo1p associated with Rpd3p and Hda1p *in vivo* and inhibited Hda1p HDAC activity *in vitro*. These data indicate that Sdo1p constitutes the first example of an endogenous antagonist of HDAC activity in yeast. Our findings also suggest that some of the pathogenic effects of SBDS deficiency in humans may relate to alterations in chromatin structure, and that HDAC inhibitors may offer therapeutic promise for treating SDS and related diseases.

An Alternative rRNA Processing Pathway Generates a Specialized Ribosome That is Used for Nonsense-Mediated Decay

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A Systematic Genetic Analysis (SGA) with mutations in the genes for the MRP RNA (*NME1*) and the protein component (*SNM1*) was performed to better understand the role of RNase MRP in cellular RNA metabolism. The L16a and b proteins differ by only 10 amino acids, but these residues cluster in the amino terminus of the protein, which is important for RNA binding. Hence, one protein preferentially binds the shorter form of the 5.8S and 25S rRNAs, while the other prefers the longer forms with the A1 helix. This suggests that a subtle difference in rRNA processing can lead to the association of a different complement of protein components and potentially confer different functions to a ribosome. The microarray analysis of an RNase MRP mutant revealed that expression is

considerably down for a number of genes whose transcript levels are regulated by NMD. This would indicate that nonsense-mediated decay of these mRNAs is overactive. The level of *CPAI* is clearly down in RNase MRP mutants, consistent with too much NMD, and as predicted by the model that deletion of one of the yeast NMD genes (*NAM1/UPF1*) recovers levels of this transcript. The high-resolution structure of the *T. thermophilus* ribosome indicates that the L17 protein stretches all the way into the protein channel of the large subunit. Hence, a premature stop in translation could be immediately transduced through the L17 protein to the L16 protein. The L16b protein has been shown to interact with the Sup35 protein, which has been shown to interact with all three NMD proteins. These results are providing an unprecedented molecular view of how a cell might promote the decay of mRNAs when protein translation is halted prematurely. Subtle changes in rRNA processing can easily promote a variety of disease states in humans.

IV. THE NUCLEOLUS AND rDNA TRANSCRIPTION

RPS19 and Diamond Blackfan Anemia

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Mutations in the gene encoding ribosomal protein S19 (*rps19*) have been identified in 25 percent of patients affected by Diamond Blackfan anemia (DBA). Recently, mutations in other genes encoding for ribosomal proteins have been found in an additional 20 percent of DBA cases. There is significant variability in the expression levels and subcellular localization of various RPS19 mutant proteins. Two distinct types of RPS19 protein defects in DBA were demonstrated: normal expression and nucleolar localization of a functionally defective protein or a marked deficiency in the expression level and failure to localize to the nucleolus of the mutant protein. The study to define the role of the proteasome in the decreased expression levels of RPS19 proteins, showed that three proteasome inhibitors, lactacystin, MG132, and bortezomib, restored the expression levels and normal subcellular localization of several unstable mutant proteins.

These findings demonstrate an important role for the proteasomal degradation pathway in regulating the expression levels of certain mutant RPS19 proteins in DBA. The transgenic mice that overexpress different mutant RPS19 proteins provided the preliminary evidence that the level of expression of mutant RPS19 is a critical determinant of the erythroid phenotype.

Pathogenesis of the Erythroid Failure in Diamond Blackfan Anemia

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To explain why erythropoiesis is so severely affected in Diamond Blackfan anemia (DBA), RNA biogenesis was measured in primary mouse fetal liver cells. During the first 24 hours, cell number increases three- to fourfold; remarkably, there is a sixfold increase in RNA content during the same period, suggesting that the cells accumulate an excess of ribosomal RNA (80 percent of measured RNA) during early erythropoiesis. The retrovirus-infected siRNA RPS19 knockdown cells show reduced proliferation of FACS-sorted GFP-positive cells at 48 hours. Although the cell yield is reduced, the differentiation pattern of the surviving GFP-positive cells is similar to that of the controls. Whereas quantitative RT-PCR analysis shows that RPS19 mRNA is rapidly depleted, Western

analysis during this time course does not show a deficiency of RPS19 protein. This suggests strongly that the proliferative defect is not due to insufficiency of RPS19 protein, and is more likely due to the block in ribosome biogenesis. With modified culture system to allow expansion without differentiation of immature cells in EPO, IGF-1, and dexamethasone, proliferation of siRNA-expressing precursors is reduced. Cell cycle analysis shows a reduced proportion of cells in S phase and an increase in G0/G1 in the knockdown cells. Erythropoietin and stem cell factor signaling leads to normal S6 kinase phosphorylation, suggesting that at least this translational pathway is intact. Preliminary data show that p53 is increased in the knockdown cells. These data suggest that RPS19-insufficient erythroid cells proliferate poorly because of inadequate accumulation of ribosome synthetic capacity. The surviving cells differentiate normally but slowly, giving rise to macrocytes. In conclusion, kinetic considerations can explain the erythroid deficiency in DBA.

V. RIBOSOMAL BIOGENESIS

Beware Imbalance in the Synthesis of Ribosomes

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Imbalance between the parts of ribosomes can lead to cell cycle arrest, to apoptosis, or to tumorigenesis. How does the cell avoid such imbalance? It's hypothesized that the coupling of rRNA transcription with the production of ribosomal proteins (RPs) occurs through the interaction of Utp22 and Rrp7 with Ifh1. These three proteins can form a complex together with CK2. It's suggested that active rRNA transcription leads to the sequestering of Utp22 and Rrp7 in processing particles, releasing Ifh1 to drive transcription at RP genes. Slowing of rRNA transcription would release Utp22 and Rrp7, which in turn would sequester Ifh1 to reduce RP gene transcription. Support for this hypothesis comes from the observation that experimental depletion of either Rrp7 or Utp22, but not of other processing factors, leads to a substantial increase in RP gene transcription in spite of severely slowed growth. A second key question is how the cell deals with an experimentally induced imbalance due to semi-starvation for a single RP. In the case of deletion of *RPL1B*, reducing by half the supply of ribosomal protein L1, the cell requires hyperactivity of the ubiquitin-proteasome system. Double mutants of *RPL1B* with many members of the ubiquitin-proteasome system have a synthetic lethal phenotype, which can be suppressed by overexpression of *UBI4*, encoding ubiquitin itself. Hyperactivity of the ubiquitin-proteasome system is suggested to be required to degrade the excess ribosomal proteins released when a misassembled ribosomal subunit is destroyed.

Physical and Functional Clusters of Ribosome Assembly Factors: Recruiting Strategies and Mechanisms to Regulate Pre-rRNA Processing

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Studies of ribosome biogenesis in the yeast *Saccharomyces cerevisiae* have revealed ~180 *trans*-acting factors required for assembly of ribosomes, most of which are essential and conserved throughout eukaryotes. The current focus is on the two consecutive steps in assembly of 60S ribosomal subunits in which a subset (25) of these assembly factors specifically functions—maturation of preribosomes,

in which processing of the 27SA3 and 27SB pre-rRNAs occurs. These 25 proteins include examples of most of the potential functions ascribed to ribosome assembly factors: RNA binding proteins, scaffolding proteins, nucleases, GTPases, and ATP-dependent RNA helicases/RNPases. The strategy is to use genetic, biochemical, and proteomic tools available in yeast, and to develop new tools to identify and understand the dynamic networks of physical and functional interactions among these 25 assembly factors, as well as their interactions with r proteins and pre-rRNAs. Identifying these interactions should reveal cofactors with which each assembly factor functions, the substrates upon which they act, and molecules that recruit them into or release them from preribosomes. This strategy is believed to be an efficient next step toward the ultimate goal to understand mechanisms of ribosome assembly.

Utp4/Cirhin as a Cause of North American Indian Childhood Cirrhosis

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North American Indian childhood cirrhosis (NAIC) was reported to be caused by a recessive missense mutation in the small subunit (SSU) processome protein, cirhin. The yeast homolog of cirhin, Utp4, is essential for cell growth and plays a role in both optimal transcription of the rDNA and in pre-rRNA processing. In order to determine whether the NAIC mutation affects ribosome biogenesis, the analogous mutation in yeast Utp4 was created. In NAIC, cirhin is mutated at arginine 565 to tryptophan (R565W). However, alignment of cirhin/Utp4 sequences from several vertebrate and invertebrate species indicates that there is a lysine, not an arginine, at the two potentially analogous positions. Both of these lysine residues were mutated to tryptophan, and the effects on ribosome biogenesis were examined. Unexpectedly, neither of these mutations resulted in any defects in ribosome biogenesis, as determined by a variety of assays. In addition, several Utp4 truncations in the region surrounding the NAIC mutation were created. These mutations show that the C-terminal region of Utp4 is important for cell growth, as truncation by as few as 31 amino acids leads to defective growth. It's hypothesized that these truncations interfere with protein-protein interactions between Utp4 and another member of the SSU processome. This possibility is currently being investigated by directed yeast two-hybrid analysis. The findings thus far are consistent with the idea that mutations in SSU processome components that cause human disease are likely to decrease, but not obliterate, protein function.

Yeast Models of Diamond Blackfan Anemia and Shwachman Diamond Syndrome Differ in Their Effect on the Synthesis and Function of Ribosomal Subunits

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Yeast models of Diamond Blackfan anemia (DBA) and Shwachman Diamond syndrome (SDS) were studied to determine potential underlying molecular mechanisms that distinguish these disease models. All genes identified in DBA encode ribosomal proteins, while *SBDS* encodes a protein that associates with 60S subunits but is not considered a structural component of the ribosome. Polysome profiles from cells depleted of *RPL33A* have a decrease in the amount of free 60S subunits and the

presence of half-mer polysomes, as expected for an essential structural component of the 60S subunit. Polysome profiles from cells depleted of *SDO1* also have half-mer polysomes, but in this case there are significant amounts of free 60S subunits evident. Further studies indicated that depleting cells of *SDO1* influences both 60S subunit biogenesis and the ability of 60S subunits to join with 40S subunits during 80S initiation complex formation. Thus, there are significant differences in the details by which the losses of *RPL33A* and *SDO1* impact the synthesis and function of 60S ribosomal subunits. To determine if these mechanistic differences influence protein synthesis, the patterns of proteins synthesized in these two disease models were analyzed. The expression of the 20S replicon was induced in both models. However, the two models also showed distinct differences in the synthesis of certain proteins. Thus, the mechanisms by which depleting cells of *RPL33A* and *SDO1* influences levels of functional 60S subunits have differential effects on the patterns of proteins synthesized within cells. These data indicate that the ribosome-based diseases may be a composite of effects that include both nucleolar stress mechanisms and changes in translational output, either of which may factor into their distinct clinical phenotypes.

VI. RIBOSOMES AND DISEASE

Clinical Features of Ribosomal Hematologic Diseases

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The inherited bone marrow failure syndromes (IBMFS) are genetic disorders with progressive bone marrow failure. The syndromes that clearly involve ribosomal assembly are Diamond Blackfan anemia (DBA) and Shwachman Diamond syndrome (SDS). One of the many genes mutated in Dyskeratosis Congenita (DC) is dyskerin, which may also play a role in ribosomal assembly. The other major IBMFS include Fanconi anemia (FA), a DNA repair disorder, severe congenital neutropenia (SCN), amegakaryocytic thrombocytopenia (amega), and thrombocytopenia absent radii (TAR). In addition to the heterogeneity of molecular mechanisms, the IBMFS have diverse physical abnormalities, including: 1) birth defects and findings that appear with age; 2) some common and some different hematologic presentations; 3) varied risks of leukemia and cancer, in terms of quantity and types; and 4) different survival times overall and after stem cell transplant. Treatment also is syndrome-specific: stem cell transplant may cure the bone marrow of aplastic anemia, myelodysplastic syndrome (MDS), or leukemia; patients with FA and DC remain at high risk of solid tumors. Medical management includes steroids in DBA; G-CSF in SDS and SCN; androgens in FA, DC, and amega; and spontaneous improvement in TAR. Diagnostic screening tests are red cell ADA in DBA, serum pancreatic enzyme levels in SDS, chromosome breakage in FA, telomere length in DC, and radiographs in TAR; sequencing of candidate genes is applicable to all disorders. Clinical examinations and routine laboratory tests are not always specific, and their relation to the underlying molecular mechanisms is not entirely clear.

Shwachman Diamond Syndrome: Clinical and Molecular Features

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Shwachman Diamond syndrome (SDS) is an autosomal recessive multi-system disorder clinically characterized by exocrine pancreatic dysfunction, bone marrow failure, increased risk for myelodysplastic syndrome and acute myelogenous leukemia, short stature as well as and other

skeletal abnormalities. Clonal marrow cytogenetic abnormalities commonly arise and frequently involve chromosome 7 and chromosome 20. The majority of SDS patients carry biallelic mutations in the *SBDS* gene. Bone marrow studies have revealed reduced hematopoietic progenitor colony formation, increased apoptosis, and impaired stromal cell function. Work by other laboratories demonstrated that RNAi depletion of Sbd protein in mouse hematopoietic cells results in impaired myeloid precursor production and differentiation, diminished numbers of circulating B cells, and reduced bone marrow homing. Abrogation of the yeast orthologue, *Ylr022c/Sdo1*, results in a slow growth phenotype that is suppressed by mutations in *Tif6*. A model has been proposed whereby Sdo1 promotes the release of Tif6 from the 60S ribosome to facilitate ribosome subunit joining, possibly together with the GTPase Efl1. Our studies in human cells also support a role for SBDS in ribosome biogenesis. Human SBDS protein shuttles in and out of the nucleolus in a regulated fashion and associates with the 60S subunit. Loss of SBDS results in diminished rRNA production. SBDS also binds and stabilizes microtubules to prevent genomic instability. The demonstration that purified recombinant SBDS protein binds and stabilizes microtubules *in vitro* supports a direct function of SBDS in this process. Thus, SBDS is emerging as a multifunctional protein involved in both ribosome production and genomic instability in an inherited marrow failure and leukemia predisposition syndrome. The relationship between these seemingly disparate functions remains an area of active investigation.

Shwachman-Diamond Syndrome Mice Display ‘Minute-Like’ Phenotypes with Translational Insufficiency

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Shwachman Diamond syndrome (SDS) patients who carry two early truncating alleles have not been described, and mice that are homozygous for null alleles (*Sbds*^{-/-}) exhibit embryonic lethality. A R126T missense disease allele was generated on the prediction that this mutation is hypomorphic. *Sbds*^{R126T/wt} mice were found to develop normally and show no disease phenotypes, in accordance with the recessive inheritance of SDS. However, both *Sbds*^{R126T/R126T} and *Sbds*^{R126T/-} embryos exhibit marked size reduction and die at birth. Growth differences become apparent in the mid-fetal period with noted delay or abnormalities of major organs, including the skeleton, brain, and lung. Hematopoiesis of the fetal liver also is disturbed, with low numbers of progenitor cells of both myeloid and lymphoid lineages. Comparable deficiencies were noted overall, but the *Sbds*^{R126T/-} embryos were consistently more severely affected than *Sbds*^{R126T/R126T} embryos. Investigations of mouse embryonic fibroblasts indicated impairment of protein translation capacity in mutant cells, as well as slow growth. Polysome profiles were also abnormal, exhibiting marked reductions in 80S monoribosomal peaks as well as an increase in the ratio of 40S to 60S subunit peaks. These cellular deficiencies, together with the developmental delay and poor growth, emphasize the severe consequences of loss of *Sbds*. These findings are reminiscent of the classic *Minute* mutations that have been described in *D. melanogaster*, and indicate that SDS is a translation insufficiency syndrome.

Diamond Blackfan Anemia: Results From the Diamond Blackfan Anemia Registry

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Diamond Blackfan anemia (DBA) is a heterogeneous genetic disorder characterized by red cell aplasia, congenital anomalies, and a predisposition to cancer. Although incompletely understood, the erythroid failure in DBA appears to result from the accelerated apoptosis of affected erythroid progenitors/precursors. Six DBA genes, coding for ribosomal proteins RPS19, RPS17, RPS24, RPL35a, RPL5, and RPL11, have been identified. Abnormal rRNA processing is a consistent feature of DBA for all described genotypes. Even within multiplex families, individuals may vary dramatically as to the degree of anemia, response to treatment, and presence of congenital anomalies. The Diamond Blackfan Anemia Registry (DBAR), a comprehensive database of pediatric and adult patients with DBA who are enrolled after informed consent, was designed to overcome two significant obstacles encountered in the study of a rare disease: the reporting bias inherent in the literature and the lack of an active patient database. To enroll patients, their families and their physicians complete a detailed questionnaire. A review of medical records and telephone interviews is performed to complete and clarify the information provided. As of August 1, 2008, 555 patients have been enrolled in the DBAR. Epidemiological, clinical, and laboratory data have been collected and analyzed. The DBAR has provided new information on the clinical presentation, outcome, and genetics of DBA, as well as a better description of congenital malformations and cancer predisposition. The DBAR has been instrumental in the identification of four of the six heretofore described DBA genes.

Ribosome Biogenesis and Bone Marrow Failure

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During the last 10 years a number of genes have been identified that, when mutated, are responsible for certain forms of inherited bone marrow failure syndromes (IBMFSs). Four of the IBMFSs have been associated with impairment of ribosome biogenesis: (1) Diamond Blackfan Anemia (DBA) is caused by mutations in genes encoding several of the ~80 ribosomal proteins; (2) X-linked and rare cases of autosomal recessive dyskeratosis congenita (DC) can be caused by mutations in dyskerin, NHP2, and NOP10, all components of Box H/ACA ribonucleoprotein particles (RNP); (3) Shwachman Diamond syndrome (SDS) is caused by mutations in SBDS, a protein implicated in a late step of ribosome synthesis; (4) Cartilage hair hypoplasia is caused by mutations in the RNase mitochondrial RNA processing gene *RMRP*.

Although patients with these disorders share some clinical features, they show characteristic differences, including the cell lineage and tissues preferentially affected and the risk and type of malignant transformation. The gene products affected participate in different steps of ribosome biogenesis; furthermore, some are involved in additional biological functions, for example, telomere maintenance, RNA cleavage, or mitosis, and thus connect ribosome biogenesis with other pathways essential for cell growth, cell proliferation, and cell survival. Studies of altered ribosome biogenesis in these IBMFSs therefore not only highlight the role of ribosome biogenesis in these rare conditions but also explore their importance in tumorigenesis and tissue regeneration. Investigations will provide new insights into basic mechanisms of cell growth, proliferation, and survival and are likely to uncover novel and unexpected rules of biology.